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Elastin: A Stimuli Responsive Biopolymer for Nano-, and Micro-Actuation

Principal Investigator: Gabriel P. Lopez

GRANT #: N00014-00-1-0183

Abstract

We developed hybrid materials comprising of silica and stimuli responsive polymers (SRPs) to demonstrate the feasibility of SRPs as actuation elements in functional nanostructured materials. We successfully demonstrated that the hybrid membranes comprising of silica and SRPs can function as molecular filters with reversible permeability and good stability. We dispersed these SRPs in a silica matrix at a molecular level using sol-gel process. The encapsulated SRP molecules to act as nano-valves whose permeability can be controlled by cycling through the lower critical solution temperature (LCST) of the SRPs. Micropatterning studies on hybrid membranes suggest that a wide variety of water-soluble species can be patterned without need for a specific chemisorption or affinity label using localized heating with a UV laser. Developed porous silica nanocomposite materials and modified the surface with SRPs using atom transfer radical polymerization and demonstrated that the size and surface energy of the pores can be externally and reversibly controlled to dynamically modulate the adsorption and transport of molecular species. These have potential applications in the areas of separations, controlled drug delivery, environmental remediation, bioassays, biosensing, tissue engineering.

Using a model system of silica and PNIPAAm we have demonstrated design principles and synthetic methods are applicable to a wide variety of porous structures and polymers (or small molecules) which are reversibly sensitive to different external stimuli such as temperature, light, electricity, pH, solutes, or enzymatic transformations.

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FINAL REPORT

GRANT #: N00014-00-1-0183

PRINCIPAL INVESTIGATOR: Prof. Gabriel P. Lopez

INSTITUTION: The University of New Mexico

GRANT TITLE: Elastin: A Stimuli Responsive Biopolymer for Nano-, and Micro-Actuation

AWARD PERIOD: 1 December 1999 – 30 November 2002

OBJECTIVE: To examine the feasibility of elastin like polypeptides (ELP) and poly(N-isopropyl acrylamide) (PNIPAAm) as nanoscopic chemo-mechanical actuators and to understand the physico-biochemical properties of these stimuli-responsive polymers (SRPs) and to demonstrate the feasibility of SRPs as actuation elements in functional nanostructured materials.

APPROACH: SRPs were encapsulated in dense silica matrices using sol-gel processing to prepare smart hybrid nanocomposite membranes. By dispersing the SRPs at a molecular level, the encapsulated SRP molecules to act as nano-valves whose permeability can be controlled by cycling through the lower critical solution temperature (LCST) of the SRPs. Molecular permeability through these hybrid materials was controlled by the extended and contracted conformation of ELPs and PNIPAAm. We exploited the thermo-responsive conformational changes of PNIPAAm chains to reversibly create pores in a silica matrix for a variety of applications which include micropatterning of dye molecules, low molecular weight polymers and peptides labeled with fluorescein.

Ordered mesoporous silica microspheres were prepared by a surfactant templating technique using evaporation induced self-assembly. PNIPAAm was grafted into the mesopores of silica matrix using the novel approach of atom transfer radical polymerization (ATRP). In this process, the life time of growing radical on the surface is high (several hours) enabling the preparation of polymers with predefined molar masses, low polydispersity, controlled compositions and functionality. A major advantage of ATRP is that the free radical is confined to the growing polymer chain, thus eliminating the problem of free radicals in solution forming bulk polymer which may in turn clog the pores. Capture and release of fluorescent probes in SRP-grafted silica microspheres were studied by flow cytometry.

Novel temperature sensitive surfaces can also be prepared by grafting SRPs on gold surfaces using ATRP. We have synthesized dense and uniform polymer brushes of PNIPAAm and copolymers of NIPAAm and *N*-tert butyl acrylamide with ATRP on gold surfaces at room temperature. The advantages of ATRP for this purpose include precise and independent control of the thickness, density and composition of the

polymer films. The transitions of these smart surfaces were studied by surface plasmon resonance (SPR), contact angle goniometry and ellipsometric techniques.

ACCOMPLISHMENTS :

Smart Hybrid Membranes: We developed molecular switches as nanoscopic actuators that can control the transport of chemical species by encapsulating ELPs or PNIPAAm in a dense silica gel. Figure 1 illustrates that the hybrid membranes could function as permeability switches upon cycling between 25°C (below LCST) and 40°C (above LCST) with good mechanical stability. Permeation experiments with monodisperse poly(ethyleneglycol) (PEGs) on silica-PNIPAAm and silica-ELP filters demonstrated that these could function as switchable molecular filters (Table 1). Figure 2 demonstrates that change of hydrophilic to hydrophobic state of hybrid membrane by changing the temperature.

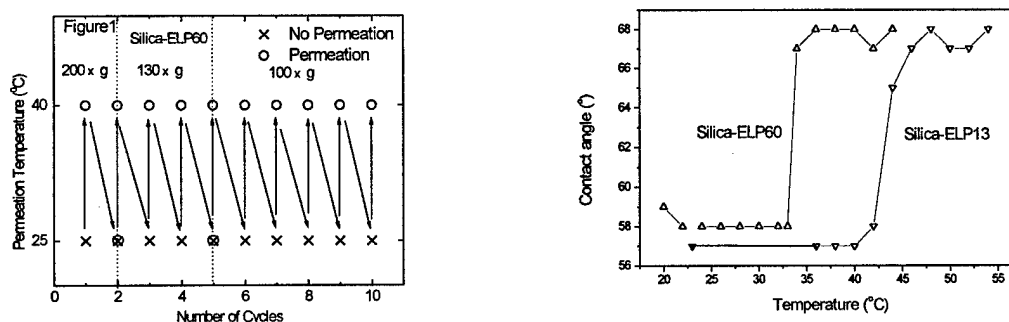


Figure 1. (left) illustrates that the hybrid membranes could function as on/off permeability switches when they cycle between 25°C (below LCST) and 40°C (above LCST).

Figure 2. (right) demonstrates that change of hydrophilic to hydrophobic state of hybrid membrane by changing the temperature.

Table 1. Permeation of poly(ethylene glycol) (PEG) solutions through silica-ELP60 (molecular weight 60 kDa) membranes coated on YM-30 centrifugal filters (Refractive index (R.I) of pure water is 1.3330.

PEG M_w (Da)	Concentration (wt%)	R.I. of feed (± 0.0002)	R.I. of filtrate (± 0.0002)	% Rejection
1000	3.5	1.3370	1.3370	0
2200	2	1.3348	1.3345	0
5000	2	1.3352	1.3328	100
7850	2	1.3355	1.3330	100
9000	3	1.3366	1.3330	100

Micro-patterning of Smart Hybrid Membranes: We utilized the conformational changes of SRPs in a silica matrix for patterning various aqueous molecular species by entrapping them in the pores created by the LCST behavior of the SRPs. This method enabled the patterning of wide variety of water-soluble species without the need for specific chemisorption or affinity labels. This process is reversible; entrapped molecules are released when the substrate is rinsed with water at 40°C. Figure 3 shows patterning of fluorescein on hybrid membranes. Fluorescein molecules are entrapped in the pores of SRP membranes due to localized heating with a UV laser. This process is reversible; entrapped molecules are released when the substrate is rinsed with water at 40°C.

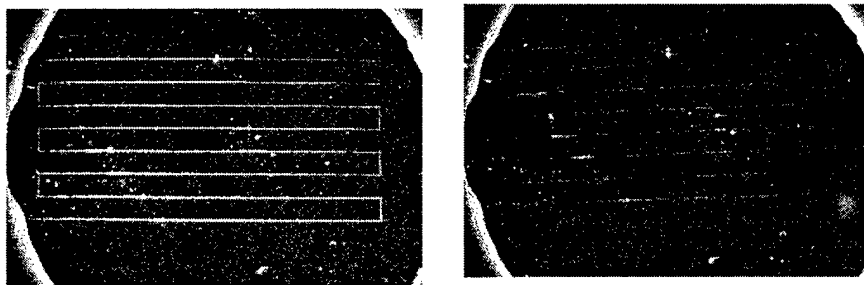


Figure 3 Left. Micrograph showing the micropattern of fluorescein on smart membrane; Right- After washing at 40°C (scale bar corresponds to 100µm).

Mesoporous Smart Hybrid Materials: We successfully grafted PNIPAAm onto mesopores of silica microspheres and demonstrated the capture and release of fluorescein molecules from the pores. Fig. 4 presents the fluorescence of dye-loaded particles as a function of time obtained from flow cytometry studies. A more rapid decrease in particle fluorescence is observed at 50°C than at 25°C, indicating that the hydrated PNIPAAm chains restrict molecular diffusion at low temperatures.

The release of rhodamine 6G dye from polymer grafted polymer particles was monitored (in situ) at 25 and 50°C by confocal microscopy. Fig. 5A presents confocal fluorescence images that show the time dependent release of rhodamine 6G at 50°C. Fluorescence profiles of the particles at various time intervals indicate (Fig. 5B) the release of the dye from the particle. Images taken at 25°C showed a much slower change in fluorescence intensity.

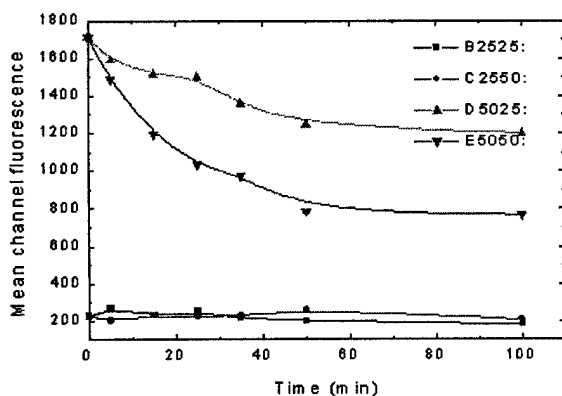


Figure 4. Release of fluorescein from PNIPAAm grafted mesoporous particles as a function of time as measured by flow cytometry. All samples were equilibrated in dye (35µM) for 2 hrs. Fluorescence of beads at 25°C was measured for beads incubated at 25°C (●) and 50°C (▲). Fluorescence of beads at 50°C was measured for beads incubated at 25°C (◆) and 50°C (▼).

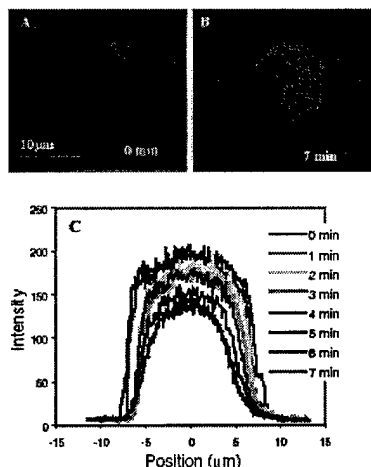


Figure 5. Confocal micrographs demonstrating the release of rhodamine 6G from the polymer grafted particles at 50°C. A. at $t = 0$ min, B. at $t = 7$ min and C. fluorescence intensity line profiles taken from the horizontal midline of the image of the particle as a function of time.

Temperature Sensitive Surfaces: NIPAAm and copolymers with *N*-tert-butylacrylamide (NTBAAm) were synthesized by ATRP technique which facilitated the successful grafting of dense and uniform polymer brushes. The thermally induced hydration transition of surface-grafted (PNIPAA) brushes was probed by surface plasmon resonance spectroscopy (SPR) (Fig.6) and contact angle measurements. The results suggest that the polymer segments in the outermost region of the brush remain highly solvated until the dilute solution lower critical solution temperature ($\sim 32^\circ$), while densely packed, less solvated segments within the brush layer undergo dehydration and collapse over a broad range of temperatures.

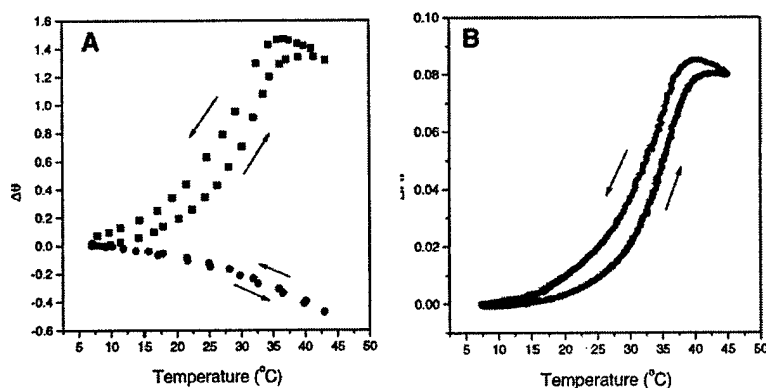


Figure 6. Temperature-dependent SPR. (A) Change in the minimum of the SPR curve ($\Delta\theta$) for the PNIPAAm layer (\blacksquare) and for the control surface (a SAM formed from 11-mercapto-undecane) (\bullet) as a function of temperature. The sample was equilibrated at every temperature for 15 min before the resonance curves were obtained. The plotted values are averages of three values. The average standard deviation in the resonance angle measurements was 0.0047 for the polymer and 0.0043 for the control. (B) Real-time measurement of change in the reflected intensity at a constant angle (74.5°) for the PNIPAAm layer as a function of temperature. The heating rate was $4.8^\circ\text{C}/\text{min}$, and the cooling rate was $4.3^\circ\text{C}/\text{min}$.

Electrochemical Actuation of Smart Polymers: Immobilization of multi-amine elastin like polypeptides(ELPs) onto mixed self-assembled monolayers was carried out through the formation of amide bonds.

CONCLUSIONS: We successfully demonstrated that hybrid membranes comprising of silica and SRPs can function as molecular filters with reversible permeability and good stability. Micropatterning studies on hybrid membranes suggest that a wide variety of water-soluble species can be patterned without need for a specific chemisorption or affinity label using localized heating with a UV laser. Developed porous silica nanocomposite materials and modified the surface with SRPs and demonstrated that the size and surface energy of the pores can be externally and reversibly controlled to dynamically modulate the adsorption and transport of molecular species. These have potential applications in the areas of separations, controlled drug delivery, environmental remediation, bioassays, biosensing, tissue engineering.

Using a model system of silica and PNIPAAm we have demonstrated design principles and synthetic methods are applicable to a wide variety of porous structures and polymers (or small molecules) which are reversibly sensitive to different external stimuli such as temperature, light, electricity, pH, solutes, or enzymatic transformations.

SIGNIFICANCE: Silica-PNIPAAm/ELP hybrid membranes can function as molecular filters, which are sensitive to the temperature changes. These can be used as nano-valves and tunable molecular sieves. The following applications are envisaged.

1. Molecular separations through selective (tunable) ultrafiltration
2. Molecular separations through selective (tunable) chromatography
3. Molecular valves and pumps in microfluidic systems
4. Controlled drug delivery
5. Tissue engineering
6. Regeneration of bioactive surfaces

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10. Fu, Q. Rao, G.V.R., Lopez, G.P. "Copolymer Membranes" (in preparation)
11. Rao, G.V.R., Petsev, D., Fu, Q., Lopez, G.P. (in preparation)
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